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Expression of the Human Epidermal Growth Factor Receptor in a Murine T-cell Hybridoma

A TRANSMEMBRANE PROTEIN TYROSINE KINASE CAN SYNERGIZE WITH THE T-CELL ANTIGEN RECEPTOR*

(Received for publication, August 8, 1991)

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Although the T-cell receptor for antigen (TCR) lacks intrinsic kinase activity, stimulation of this receptor induces tyrosine phosphorylation of multiple substrates. In contrast, the epidermal growth factor receptor (EGFR) has intrinsic cytoplasmic tyrosine kinase catalytic activity that is activated upon EGF binding. To compare the functional effects of the TCR and a transmembrane protein tyrosine kinase (PTK), we used retrovirus-mediated gene transduction to express the human c-erbB proto-oncogene, encoding the EGFR, in a murine T-cell hybridoma. Tyrosine phosphorylation induced by the TCR and the EGFR occurred on substrates unique to each receptor as well as on several shared substrates, including the ζ chain of the TCR. Stimulation of the EGFR induced calcium ion flux in these cells, suggesting that the heterologous tyrosine kinase can couple to the T-cell phospholipase signal transduction pathway, but this stimulus did not lead to interleukin 2 production. However, EGF stimulation of transduced cells significantly enhanced TCR signaling, as assessed by interleukin 2 production, indicating that cross talk can occur between the TCR and a transmembrane PTK.

The earliest recognizable event following engagement of ligand by the T-cell receptor for antigen (TCR)[1] is rapid tyrosine phosphorylation of multiple cellular substrates (1). The mechanism by which these phosphorylations are induced is not known. One possibility is that the TCR, which lacks intrinsic kinase catalytic activity, is able to couple to a non-receptor protein tyrosine kinase (PTK) of the Src family, and that this kinase is then responsible for subsequent phosphorylations. We have previously expressed the constitutively active kinase *v-src* in T cells and demonstrated that this can mimic some aspects of TCR mediated T-cell activation (2). At present $p56^{lck}$ and $p59^{fyn}$ are the two most favored candidates for the role of TCR-associated kinase (3). When T-cells recognize antigen in the context of MHC, CD4, or CD8, which associate with $p56^{lck}$ (4, 5), and the TCR, are localized in the region of cell contact (6). This co-receptor relationship may thus allow $p56^{lck}$ to mediate TCR-directed tyrosine phosphorylations. Demonstration of a physical association between $p59^{fyn}$ and the TCR in a T-cell hybridoma raises the possibility that this may also be a TCR coupled PTK (7). Supporting this hypothesis is the observation that thymocytes from transgenic mice overexpressing $p59^{fyn}$ show enhanced TCR-mediated signaling (8).

The mechanism by which a signal is transmitted from the TCR to a receptor coupled PTK is not known. In contrast, signaling by the product of the c-erbB proto-oncogene, the epidermal growth factor receptor (EGFR) (9), is better understood. Binding of EGF by its receptor is accompanied by conformational changes in the extracellular domain of the latter with receptor dimerization (10). This is hypothesized to permit the cytoplasmic PTK domains of each member of the dimer to come into close proximity effecting receptor tyrosine kinase activation (11).

Recent data have suggested a parallel between the intracellular events and second messenger systems involved in signaling by the TCR and transmembrane protein kinases such as the EGFR or platelet-derived growth factor receptor. For instance, the EGFR and TCR initiate tyrosine phosphorylation as a primary event (1, 11), with subsequent phospholipase C activation and calcium ion flux (1, 12-14). Both receptors are phosphorylated on tyrosine (11, 15) and serine (16, 17) as a consequence of activation. The TCR can associate with a Src family kinase (7), and the platelet-derived growth factor receptor is induced to do so upon activation (18). Stimulation of the TCR and EGFR promotes accumulation of $p21^{ras}$ in its active GTP bound form (19, 20), increases expression of the transcriptional regulators fos and myc (21-23), and induces phosphorylation of Raf-1 kinase (24, 25).

To directly compare the intracellular events mediated by the TCR and a transmembrane PTK, we used retrovirus-mediated gene transduction to express the human EGFR in the murine T-cell hybridoma 2B4.11 (26). We compared the tyrosine phosphorylation events and calcium ion flux resulting from activation of both receptors in this system. Upon ligand binding the introduced receptor induced phosphorylation of multiple substrates on tyrosine. On IEF/SDS-PAGE analysis, many of these substrates appeared identical to those that are phosphorylated in response to anti-CD3 and Thy-1 stimulation, suggesting that some intracellular signaling pathways may be common to the EGFR and TCR. Interestingly, the EGFR was able to induce tyrosine phosphorylation of the TCR ζ chain. Stimulation of the EGFR lead to calcium ion flux in these cells, suggesting that the EGFR can couple to

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[1] The abbreviations used are: TCR, T-cell receptor for antigen; EGFR, epidermal growth factor receptor; PTK, protein tyrosine kinase; IEF, isoelectric focusing; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; IL-2, interleukin 2; PBS, phosphate-buffered saline; PMA, phorbol 12-myristate 13-acetate.

the T-cell phospholipase signal transduction pathway. Most notably, EGFR occupancy alone was not sufficient to induce IL-2 production by these cells, but was able to significantly enhance TCR signaling, as measured by interleukin 2 production. This raises the possibility that cross talk may occur between the TCR and transmembrane tyrosine kinases expressed on T-cells, such as the insulin receptor and insulin like growth factor receptor (27, 28).

Our model system suggests that a substantial degree of degeneracy exists between the signaling systems of the TCR and EGFR and by analogy possibly other transmembrane PTKs as well. By allowing identification of substrates that are unique to both types of receptor these cells provide an exciting means to investigate the level at which specialization of signaling occurs.

MATERIALS AND METHODS

Antibodies and Reagents—The following were purchased: recombinant human EGF (Boehringer Mannheim), EGFR monoclonal antibody Abl (Oncogene Science), anti-phosphotyrosine monoclonal antibody 4G10 (Upstate Biotechnology). Affinity-purified anti-phosphotyrosine polyclonal antiserum (29) against autophosphorylated v-*abl* kinase was a gift of Dr. L Samelson (National Institute of Child Health and Human Development).

Vector Construction and Cells—The pMNC eukaryotic expression vector carrying the neo resistance gene was provided by Dr. B. Seed and A. Peterson (Massachusetts General Hospital) (30) The plasmid pLSX carrying a human EGFR cDNA was provided by Dr. J. Schlessinger, New York University (31). A pMNC-EGFR construct was created by isolating an *Xho*I fragment containing the EGFR cDNA from pLSX and ligating this into the *Xho*I site of pMNC. The ψ2 packaging cell line (provided by D. Miller) (32) was transfected with pMNC-EGFR and screened for viral titer. Irradiated ψ2-MNCEGFR cells were then co-cultured with 2B4.11 antigen-specific murine T-cell hybridoma cells (26) and G418-resistant clones established by limiting dilution as described (32). Flow cytometry confirmed EGFR expression by multiple clones. The TCR levels of EGFR expressing cells were assessed by flow cytometry and results of experiments are reported for a clone, 2B4 EGFR.5D6 (5D6), with surface TCR comparable with parental cells (not shown). The validity of experimental findings was confirmed in other clones (not shown). A431 is a human epidermoid carcinoma cell line expressing high levels of wild type EGFR (33).

Cell Stimulation, Solubilization, Immunoprecipitation, and Electrophoresis—Prior to experiments 5D6 cells were stimulated with 0.5 μM sodium butyrate for 16 h to enhance surface EGFR expression (34). Cells were washed in serum-free synthetic medium (AIM V, GIBCO), free of EGF or TGFα, and rested in this medium for 1 h on ice. Cells were incubated with EGF (at 100 ng/ml) or antibody (an equal number of LK 35.2 cells with or without anti-CD3-ϵ antibody 2C11 (35), as a 1:20 dilution of culture supernatant; or anti-Thy-1 antibody G7 (36), as a 1:50 dilution of ascites). Stimulation was terminated by addition of PBS containing 400 μM sodium orthovanadate and 2 mM EDTA at 0 °C. Cells were then lysed, electrophoresed, and immunoblotted as described (37). For experiments involving immunoprecipitation, lysate was incubated with Abl or anti TCR-α antibody A2B4-2 (38) preadsorbed onto staphylococcal protein A-agarose and processed as described (37).

Measurement of $[Ca^{2+}]_i$ in Single Cells by Digital Image Analysis—A fluorescence digital image processing system was used to detect changes in $[Ca^{2+}]_i$ in fura-2-loaded cells. The system is similar to that described previously by Tsien and co-workers (39). Hardware consisted of a FD5000 image processor (Gould, Fremont, CA), a Zeiss Axiovert microscope, and a filter changer (Ludl Electronic Products, Scarsdale, NY) with excitation filters centered at 340 ± 10 and 380 ± 10 nm. The image processor and filter changer are interfaced to a Microvax host computer (Digital Equipment Corporation, Maynard, MA). Images were acquired at 30/s through a CCD camera (Cohu) and image intensifier (Videoscope) and processed according to code kindly provided by RY Tsien. Cells were loaded in 3 μM fura-2 AM (Molecular Probes) at 30 °C for 30 min in the presence of 0.0175% F-127; this permitted uniform loading without detectable compartmentation of the fura-2. Cells were analyzed in a chamber maintained at 36 ± 1.5 °C containing 2 ml of Hanks' buffered saline solution and 0.1% fetal calf serum using a Zeiss 40× objective with a 1.3 numerical aperture. Image acquisition was accomplished by acquiring 16 frames at each excitation wavelength, with background subtraction and shading correction as described (39). Images (512 horizontal × 484 vertical pixels at 8 bits/pixel) were acquired and stored digitally at 15-s intervals. Results are reported as the mean change in $[Ca^{2+}]_i$ of 50 cells/field.

IL-2 Assay—Cells were pretreated with sodium butyrate and resuspended in fresh medium at 0.5 × 10^6 cells/ml Cells were then stimulated or not with EGF 100 ng/ml at 37 °C. After 5 min cells were transferred to 24-well plates and further stimulated with 2C11 antibody (preabsorbed to plates for 24 h at 4 °C), G7 antibody (as a 1:50 dilution of ascites), or PMA (10 ng/ml). After 20 h, culture supernatants were harvested and frozen IL-2 measurement was performed in triplicate by measuring [^{3}H]thymidine incorporation by the IL-2-dependent CTLL cell line in response to serial dilutions of culture supernatant and comparing this with a standard curve. Control samples consisting of medium containing EGF alone or in combination with 2C11 or G7 did not support CTLL growth (not shown).

RESULTS

Functional Expression of EGFR in a T-cell Hybridoma—We used retrovirus mediated gene transduction to express a human EGFR cDNA coupled to the neomycin resistance gene in the murine antigen specific T-cell hybridoma 2B4.11. Multiple G418-resistant clones were derived and screened for surface EGFR and TCR expression using flow cytometry. For clarity, the results of subsequent experiments are reported for one clone, designated 2B4EGFR.5D6 (5D6), that expressed significant levels of surface EGFR (Fig. 1) and TCR levels comparable with 2B4.11 (not shown). Flow cytometry (Fig. 1) and binding assays with radiolabeled human EGF (not shown) did not detect EGFR on cells transduced with expression vector lacking the EGFR cDNA. Enhanced expression of transfected genes can be induced by sodium butyrate (34). Exposure of 5D6 cells to 0.9 μM butyrate for 16 h increased modal log channel fluorescence for EGFR from 1.7 to 2.8 (Fig. 1). In subsequent experiments cells were therefore preincubated with sodium butyrate to maximize receptor expression. Anti-phosphotyrosine immunoblot analysis of receptor immunoprecipitates was used to determine if the introduced gene product retained PTK activity. As shown in Fig. 2*A*, intense EGF-dependent phosphorylation of EGFR in control A431 cells and in 5D6 cells was seen. EGF stimulation of 2B4.11 cells was examined. This stimulus did not result in detectable phosphorylation of any substrates (Fig. 2*B*). Similarly, cells transduced with expression vector lacking the EGFR cDNA did not respond to EGF (not shown). In cells expressing wild type receptor, stimulation of the EGFR is

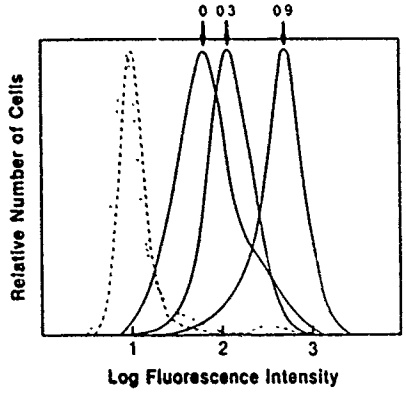

FIG. 1. **The human EGFR is expressed on the surface of 5D6 cells.** 2B4.11 infected with control plasmid lacking the EGFR cDNA (····) or 5D6 cells (——) pretreated with 0, 0.3 μM, or 0.9 μM sodium butyrate for 16 h as indicated were incubated with Abl, followed by fluorescein-conjugated goat anti-mouse F(ab')$_2$ fragments and examined by flow cytometry. 5D6 cells incubated with control antibody are shown (- - - -).

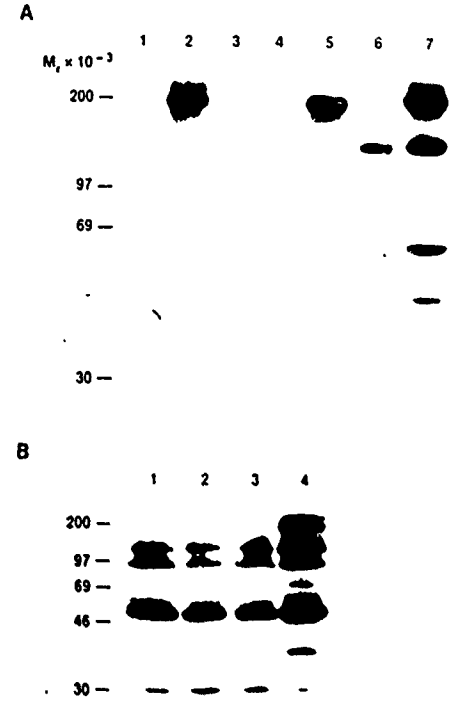

FIG. 2. *A*, the transfected EGFR autophosphorylates and phosphorylates multiple cellular substrates. 5×10^7 5D6 cells (*lanes 1–3*) and 5×10^6 A431 cells (*lanes 4* and *5*) were stimulated (*lanes 2, 3,* and *5*) or not (*lanes 1* and *4*) with EGF 100 ng/ml for 5 min, washed in PBS containing phosphatase inhibitors, lysed, and immunoprecipitated with Abl (*lanes 1, 2, 4,* and *5*) or control (*lane 3*). Total cellular lysate equivalent to 5×10^6 cells from experimental points 1 and 2 was run in *lanes 6* and *7*, respectively. After SDS-PAGE and Western transfer the filter was immunoblotted with anti-phosphotyrosine polyclonal antiserum followed by ^{125}I-SPA. *B*, human EGF does not induce tyrosine phosphorylation in 2B4.11 cells. 1×10^7 2B4.11 cells (*lanes 1* and *2*) or 5D6 cells (*lanes 3* and *4*) were stimulated (*lanes 2* and *4*) or not (*lanes 1* and *3*) with EGF 100 ng/ml for 5 min, washed in PBS containing phosphatase inhibitors, and lysed. Total cellular lysate was subjected to SDS-PAGE, Western-transferred, and immunoblotted with anti-phosphotyrosine polyclonal antiserum.

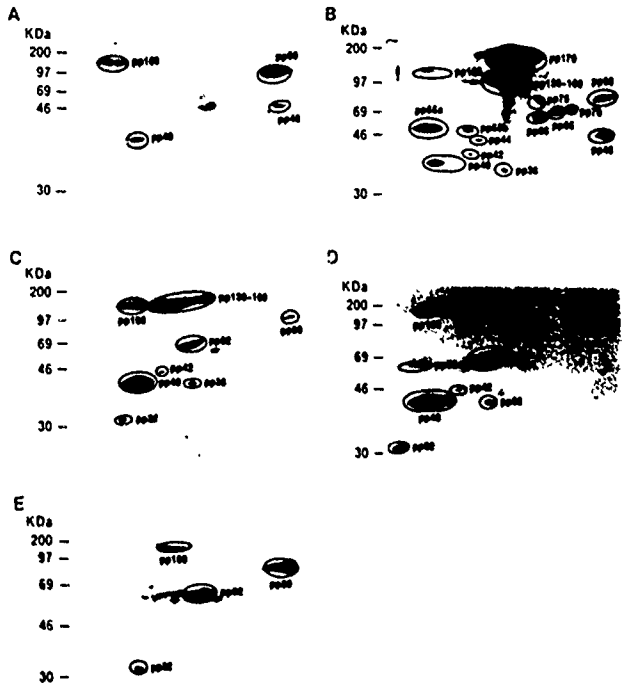

FIG. 3. **Two-dimensional IEF/SDS-PAGE comparison of EGFR-, Thy-1-, and CD3-phosphorylated substrates.** 1.5×10^7 5D6 cells were unstimulated (*A*) or stimulated with EGF (100 ng/ml, 5 min, *B*) anti Thy-1 antibody (G7 1:50 dilution of ascites, 30 min, *C*) or anti-CD3 antibody (2C11 1:20 dilution of tissue culture supernatant with an equal number of LK35.2 cells, 30 min, *D*). 1.5×10^7 LK35.2 cells alone are shown in *E*. Following stimulation, samples were processed and IEF/SDS-PAGE of whole cell lysates carried out as described (37). Blotting was with anti-phosphotyrosine monoclonal antibody.

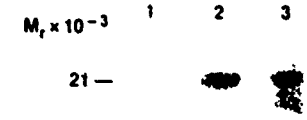

FIG. 4. **EGF stimulation induced tyrosine phosphorylation of TCR ζ.** 5D6 cells were unstimulated (*lane 1*) or stimulated with EGF 100 ng/ml for 5 and 30 min (*lanes 2* and *3*), washed with PBS containing phosphatase inhibitors, lysed, and immunoprecipitated with anti-TCR antibody A2B4-2. Immunoprecipitates were run on 13% SDS-PAGE, Western-transferred, and immunoblotted with anti-phosphotyrosine polyclonal antiserum.

known to induce rapid and transient substrate tyrosine phosphorylation (11). In EGF-stimulated 5D6 cells maximal phosphorylation of most substrates occurred 8 min after activation (not shown).

Comparison of EGFR- and TCR-dependent Tyrosine Phosphorylation—Following activation, the EGFR kinase mediates phosphorylation of multiple cellular substrates on tyrosine (reviewed in Ref. 17). Anti-phosphotyrosine immunoblots of 5D6 total cellular lysates without (Fig. 2*A*, *lane 6*) and with (*lane 7*) EGF stimulation revealed tyrosine phosphorylation, in addition to the EGFR, of several other substrates. One-dimensional SDS-PAGE comparison of these substrates to those induced by TCR stimulation revealed a potential similarity (not shown), prompting us to pursue this observation further using two-dimensional IEF/SDS-PAGE. The results are shown in Fig. 3. The brisk and intense phosphorylation of multiple proteins upon EGF stimulation was confirmed, and two-dimensional analysis revealed an additional level of substrate complexity. The M_r 170,000 species, pp170, is most likely the autophosphorylated EGFR. Beneath this is a basic substrate that is phosphorylated constitutively, pp150, and a broad band of induced substrate designated here as pp130–160. This band was intensely phosphorylated in the Thy-1 and anti-CD3 stimulated panels as well. The basal and stimulated states of phosphorylation of the acidic species pp80 and pp46 varied between experiments. The species designated pp60, pp65, pp70, and pp75 were intensely phosphorylated in the EGF-stimulated cells but were not seen with the other stimuli. This also applied to the basic substrates pp44 and pp55b. The substrates designated pp38, pp40, and pp42 were shared by EGF-, Thy-1, and anti-CD3-stimulated cells, conversely some species phosphorylated with Thy-1 and CD3 stimulation were not seen with EGF: pp32 and pp62.

EGF-mediated Phosphorylation of TCR ζ—To date, identification of substrates that are tyrosine phosphorylated upon TCR stimulation has been largely unsuccessful with the exception of ζ (40). The similarity of tyrosine-phosphorylated substrates noted above prompted us to investigate the possibility of EGFR-induced ζ phosphorylation in 5D6 cells. EGF stimulation of 5D6 cells did indeed induce phosphorylation of ζ (Fig. 4), although in contrast to the relatively delayed kinetics seen in TCR-stimulated 2B4.11 (37), the time course of EGF-induced ζ phosphorylation mimicked the transient

nature of the overall pattern of EGF-induced substrate phosphorylation.

EGF-induced Calcium Ion Flux—One consequence of EGF stimulation of cells expressing wild type receptor is a rapid increase in intracellular calcium (41). To determine if EGFR stimulation in T-cells transduced with this receptor had a functional consequence beyond tyrosine phosphorylation, we measured the intracellular calcium concentration in individual cells following EGFR and TCR stimulation As shown in Figure 5, EGF stimulation of 5D6 cells initiated a rapid rise in intracellular calcium with kinetics similar to those observed following TCR stimulation. The plateau value obtained when averaged over multiple cells was less for EGFR than TCR stimulation. When individual cells were observed the magnitude of response was similar with EGFR and TCR stimulation; the lower mean value for EGF stimulation was due to a proportion of nonresponders within the EGF-treated population (not shown).

EGF Stimulation Enhanced TCR mediated Signaling—The identification of shared tyrosine kinase substrates and EGFR-mediated calcium flux prompted us to investigate if EGFR occupancy was sufficient to induce IL-2 production and to determine if cross-talk could occur between the EGFR and the TCR. As predicted, EGF stimulation of parental 2B4 cells was without effect and no synergy occurred between EGF and TCR-mediated signals (not shown). Surprisingly, EGF alone or in combination with phorbol ester failed to induce IL-2 production by 5D6 cells (Table I). However, when 5D6 cells treated with EGF were transferred to plates bearing adsorbed 2C11 antibody, IL-2 production was significantly enhanced over 5D6 cells treated with antibody alone (Fig. 6) Similarly, EGF treatment enhanced subsequent anti-Thy-1 monoclonal antibody G7 stimulated IL-2 production by these cells (Table I).

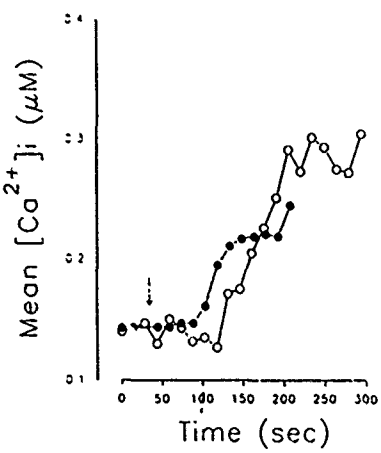

FIG. 5. **EGF-induced calcium flux in 5D6 cells.** The time course of calcium mobilization by 5D6 cells in response to 2C11 (○) or EGF (●) was analyzed by loading cells with fura-2 and measuring the $[Ca^{2+}]_i$ in individual cells by digital image microscopy. Addition of the stimuli is indicated by the *arrow*. Results are reported as mean $[Ca^{2+}]_i$ from 50 individual cells.

TABLE I

IL-2 production by 5D6 cells

Methods: 5D6 cells pretreated with 0.5 μM sodium butyrate for 8 h were resuspended in fresh medium at 0.5×10^6 cells/ml then stimulated or not with EGF 100 ng/ml. After 5 min cells were transferred to 24-well plates and further stimulated as indicated. After 20 h supernatants were assayed for IL-2 as detailed under "Materials and Methods." Data shown are representative of five experiments

Stimulus	IL-2
	units/ml
0	2
EGF	2
G7	34
G7 + EGF	52
PMA	12
PMA + EGF	9

DISCUSSION

We have expressed a functional transmembrane tyrosine kinase, the EGFR, in a murine T-cell hybridoma and used antiphosphotyrosine immunoblotting, intracellular calcium measurement, and IL-2 production to characterize the events occurring following stimulation of this receptor. We have compared these events with those occurring following stimulation with antibodies to Thy-1 and the TCR and detected both differences and similarities. Most notably we have demonstrated the ability of EGFR stimulation to augment TCR-mediated IL-2 production.

Two-dimensional Western blot analysis revealed several substrates of identical molecular weight and isoelectric point to the phosphorylated by EGFR-, Thy-1-, and TCR-mediated signals. In fact, almost all of the tyrosine phosphorylation events, as detected by us, induced by Thy-1 stimulation (which depends on the TCR for competent signal transduction (42)) were reproduced in EGF-stimulated cells. The degree of overlap of tyrosine-phosphorylated substrates indicates that substantial degeneracy may exist with respect to the signaling pathways utilized by the EGFR and TCR. Despite this degeneracy these receptors clearly must retain the ability to transmit their own unique signals; the demonstration of substrates not shared by both classes of receptor suggests one mechanism by which specialization of signaling is achieved.

It is noteworthy that EGF stimulation resulted in phosphorylation of ζ; it is not possible, however, to deduce if this phosphorylation was mediated by the EGFR as a primary event or a consequence of EGFR phosphorylation and activation of a second PTK. ζ phosphorylation has been demonstrated to occur in T-cells overexpressing the fyn (43, 44) and v-*src* (2) protein tyrosine kinases and following TCR (40), Thy-1 (45), CD2 (46), CD4 (47), CD8 (48), and CD16 (49, 50) stimulation. This is the first demonstration of ζ phosphorylation induced by a transmembrane receptor (non-*src* family) PTK and raises the possibility that ζ may not only be phosphorylated by PTK directly involved in mediating events initiated by the above receptors but accept phosphate from other transmembrane receptor PTKs expressed on T cells, (insulin receptor (27), insulin-like growth factor receptor (28),

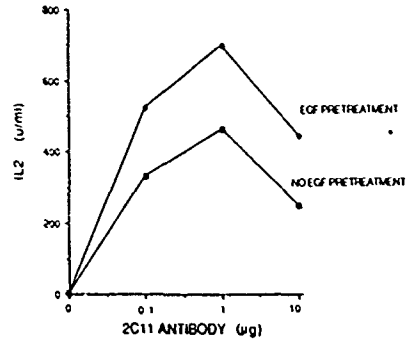

FIG. 6. **EGF-enhanced IL-2 production.** 5D6 cells pretreated with 0.5 μM sodium butyrate for 8 h were resuspended in fresh medium at 0.5×10^6 cells/ml and then stimulated or not with EGF 100 ng/ml. After 5 min cells were transferred to 24-well plates preadsorbed with the indicated dose of 2C11 antibody. After 20 h, supernatants were assayed for IL-2 as detailed under "Materials and Methods." Data shown are representative of two experiments.

platelet-derived growth factor receptor (51)), as well.

Previous studies have demonstrated that EGF can modulate immune effector cells. EGF is not mitogenic for T-cells in the absence of antigen; however, at physiologic concentrations it is able to increase antigen driven T-cell proliferation by increasing MHC class II expression on antigen presenting cells, and EGF can regulate interferonγ production by spleen cell cultures (52, 53). We reasoned that if the EGFR could be expressed in an active form in T-cells, it may transduce a signal leading to T-cell activation. Given the degree of overlap of tyrosine-phosphorylated substrates and the comparable calcium ion flux induced by the TCR and the EGFR, we were surprised that EGF stimulation of 5D6 cells did not lead to IL-2 production (Table I), even in experiments involving up to 72 h of stimulation (not shown). This observation was tested and confirmed in other EGFR expressing clones, and 5D6 cells produced levels of IL-2 similar to 2B4 cells in response to TCR or pharmacologic stimulation (not shown). Our finding is even more surprising in light of the previous observation that phospholipase activation alone (in Jurkat cells expressing the G protein-coupled and tyrosine kinase-independent muscarinic subtype 1 receptor) is sufficient to initiate IL-2 production (54). The reason for this discrepancy is unclear; it may indicate that one of the substrates phosphorylated upon TCR stimulation, but not EGFR stimulation, is a critical second messenger in the pathway leading to IL-2 production. Moreover, our data suggest that the previous demonstration that phosphatidyl-inositol pathway activation alone is sufficient for T-cell activation in Jurkat cells (54) may not be generalizable across all T-cell systems.

Despite the inability of EGF to induce even minimal IL-2 secretion when used alone, EGF treatment of transduced cells significantly enhanced anti-TCR- and anti-Thy-1-stimulated IL-2 production, indicating that cross-talk can occur between the TCR and a transmembrane PTK. Our identification of shared tyrosine-phosphorylated substrates indicates that this is one level at which cross talk is occurring. Interleukin 2 production is dependent upon the coordinate expression of multiple transcription factors (55), thus it is also possible that signals from the two receptors could be integrated at the level of the responsive gene. In this model the EGFR could, by using pathways common to, or independent of, those of the TCR, induce one or more of the factors acting on the IL-2 enhancer. This induction could then have a facilitative effect on signals subsequently transmitted by the TCR.

Finally, the demonstration of some functional similarities between the EGFR and the TCR raises the possibility that these receptors may utilize common motifs for signal transduction. Chimeric molecules formed between ζ and CD4 or CD8 are able to transmit a signal similar to that of the TCR, suggesting that ζ is central to TCR-mediated signaling (56, 57). The role of tyrosine phosphorylation of ζ in this signaling, however, remains unclear. It is known that the activated EGFR combines with a number of cellular proteins (58). In the case of some of these proteins this association probably occurs as a consequence of an avidity of their Src homology 2 (SH2) domains for the autophosphorylated receptor (59, 60). Despite the difference in kinetics (tyrosine phosphorylation of the EGFR is much more rapid than that of ζ), it remains possible that tyrosine-phosphorylated ζ could similarly combine with some or all of these molecules (phospholipase, GTPase-activating protein, phosphatidylinositol kinase, *src* family kinase, Raf-1) to form an analogous signaling complex (58). EGFR-expressing T-cells will aid in the testing of this hypothesis. Furthermore, by highlighting those substrates unique to the TCR, these cells may allow a more directed analysis of tyrosine-phosphorylated substrates important in TCR-mediated signaling and help define the level at which specialization of signaling (TCR *versus* transmembrane PTK) occurs.

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